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Investigation of Fluoroquinolones, Sulfonamides and Macrolides in Long-Term Wastewater Irrigation Soil in Tianjin, China

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Abstract Fluoroquinolones, sulfonamides and macrolides were analyzed in soil samples collected from suburban areas in Tianjin, China. Fluoroquinolones were the predominant class of antibiotics. The mean total concentration of fluoroquinolones (33.56 μg/kg) was 30-fold higher than one of macrolides, which were the second dominant class antibiotics. Sulfonamides showed the lowest level in soil. The spatial distribution of fluoroquinolones showed wastewater irrigation was a potential source of antibiotics. The individual antibiotics levels were all below the trigger value (100 μg/kg) set by the Steering Committee of Veterinary International Committee, indicating the low risk for organisms in the agricultural soils of Tianjin.

Keywords Fluoroquinolones · Sulfonamides · Macrolides · Soil

Antibiotics are important groups of pharmaceuticals in our daily lives, which are regarded as "pseudo-persistent" contaminants because they are introduced into the environment, continually. They are widely used to treat disease and protect the health of human and animals and also used as growth promoters to enhance growth and improve feed efficiency in healthy livestock. However, most of antibiotics are excreted unchanged through faeces and urine because they are poorly absorbed in the gut of the animals and not completely metabolized in the body of human or animals (McArdell et al. 2003). Antibiotics were also discharged into the environment in unchanged form via wastewater treatment plants, where they can't be eliminated completely. And antibiotics are usually still biologically active after they are discharged into the environment (Hu et al. 2010). Therefore antibiotics abuse and residue can result in the emergence of antibiotics resistance genes, a new threat to public health, which has been found in various environments, such as sediments, soil, wastewater and drinking water and so on (Knapp et al. 2010). So in recent years, there has been a growing concern about the occurrence and environmental fate of antibiotics in the aquatic and terrestrial environment.

Antibiotics are often excreted by the animals and end up in soils via grazing livestock or manure used as agricultural fertilizer. So soil is deemed to be the primary sink of antibiotics, which can persist in soil for a long time. In recent years, there are many reports on the occurrence and fate of antibiotics in the manure and manure fertilized-soil because manure and sludge applied to farmlands are often considered as major sources of antibiotics into the agricultural soil (Golet et al. 2003; Martínez-Carballo et al. 2007; Karci and Balcioğlu 2009; Li et al. 2011). In addition, wastewater irrigation was considered as another important exposure route for the introduction of pharmaceutical compounds to the agricultural fields. And antibiotics were proved to be durable contamination in the soils after cessation of long-term waste water irrigation

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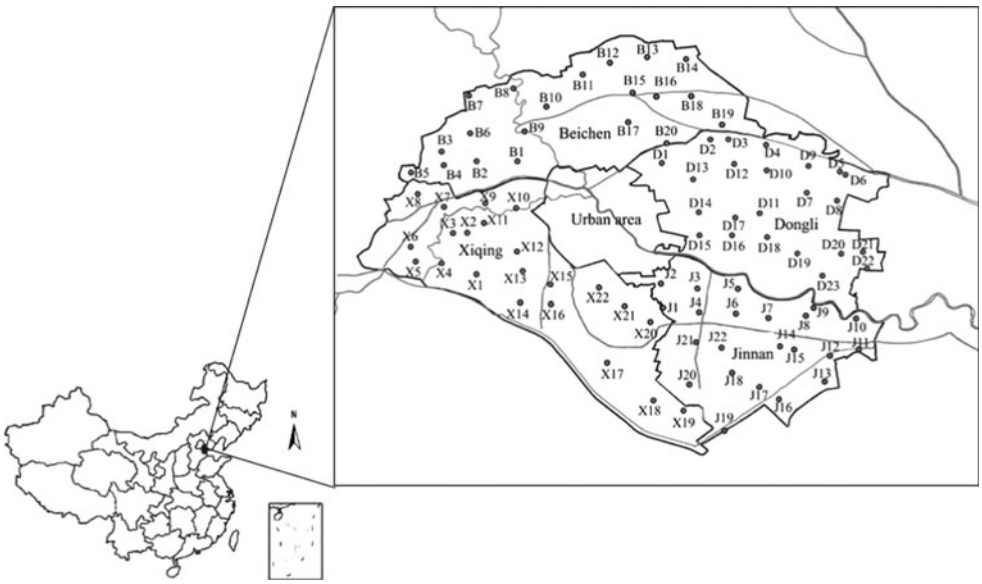

Fig. 1 Sampling sites

(Tamtam et al. 2011). However, water reclamation and reuse has become increasingly important and sustainable approaches in agricultural irrigation because of water resource scarcity and world growing demands for water (Jimenez and Chavez 2008). Especially in China, water resource scarcity is a more serious problem. Tianjin is one of the most serious water resources shortage cities, where water reclamation and reuse are common approach to solve the water shortage problem and there is the largest wastewater irrigation area in China. It has been proved that the concentrations of heavy metals in the agricultural soil of Tianjin increased due to wastewater irrigation (Shi et al. 2010). However, there is limited information on the occurrence of antibiotics in the wastewater irrigation soil in Tianjin, China. Therefore, in the present study we selected Tianjin city as the objective field to investigate the occurrence of three classes of antibiotics, fluoroquinolones (FQs), macrolides (MCs) and sulfonamides (SAs), in the wastewater irrigation soil. Both the spatial distribution of selected antibiotics and the potential effect of wastewater irrigation were discussed. To our knowledge this is the first systematical study on the antibiotic contamination in wastewater irrigation soil in China. This study provided fundamental data on the antibiotics occurrence in the wastewater irrigation soil, which are needed for risk assessment for both humans and the environment in the future.

Materials and Methods

In Tianjin city, there are three main agricultural fields irrigated using wastewater from Bei Tang Ditch, South Ditch, and Beijing Ditch. The wastewater irrigation fields mainly distribute in Dongli, Xiqing, Jinnan, and Beichen. So total of 87 soil samples were collected from the four districts, including 22 in Xiqing, 23 in Dongli, 20 in Beichen and 22 in Jinnan, in August 2008 (as shown in Fig. 1). The 0–10 cm top soil samples were collected with stainless steel scoops and stored in PE bags. Then they were transferred to the laboratory as soon as possible. After that, the soil samples were freeze-dried and sieved before analysis. They were kept at −20°C until further analysis.

One gram of each sample was spiked with 20 ng internal standard and then put into a 50 mL polypropylene (PP) centrifuge tube. The mixture sample was extracted with 15 mL of buffer solution, which is a mixture of acetonitrile and EDTA-McIlvaine buffer (pH ≈ 4.0), by sonication for 4 min, followed by centrifugation. This process was repeated twice. The extract was combined and concentrated to about 1 mL under nitrogen gas. Concentrated extracts of each sample were diluted to about 100 mL using ultra-pure water and loaded onto a HLB single-use cartridge (6 cc/200 mg) at a flow rate of 1 mL/min for cleanup. All samples were analyzed for 22 antibiotics, which included eight FQs [ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP), sarafloxacin (SAR), fleroxacin (FLE), lomefloxacin (LOM), difloxacin (DIF), enrofloxacin (ENR)], nine SAs [sulfadiazine (SDZ), sulfamerazine (SMR), sulfadimethoxine (SDM), sulfisoxazole (SIA), sulfamonomethoxine (SMM), sulfathiazole (ST), sulfapyridine (SPD), sulfamethoxazole (SMX), and sulfamethazine (SDMD)] and five MCs [erythromycin (ERY), roxithromycin (ROX), josamycin (JOS), tylosin (TYL), and spiramycin (SPI)], using high performance liquid chromatography with electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) in positive-ion mode with multiple reactions monitoring (MRM).

The detailed information on chemicals, sample cleanup, analysis and QA/QC was provided in the "Electronic Supplementary Material".

Results and Discussion

In the present study, three groups of antibiotics, FQs, SAs, and MCs, were investigated in soils from the wastewater irrigation field in Tianjin, China, and 22 antibiotics were all detectable in samples. The result showed that FQs were the predominant class of antibiotics, with the total concentrations of FQs (∑FQs) ranging from <LOD to 274.81 μg/kg. The mean concentration of ∑FQs (33.56 μg/kg) was 30-fold higher than the one of ∑MCs, which was the second dominant class of antibiotics in our study. The concentrations of ∑MCs were in the range of <LOD to 29.33 μg/kg. As indicated by Picó and Andreu (2007), FQs tend to accumulate in soils and sediments due to their strong adsorption in the soil. And experimental studies have showed that FQs can persist in agricultural soils because biodegradation (or photo transformation) of FQs is not complete in soil (Golet et al. 2003). So partition onto the solid could be the main reason for that FQs showed the highest level in the soil. On the contrary, SAs, with low K_d values (0.9–18.1 L/kg), defined as the ratio of the concentrations of an SA in soil and water at equilibrium, have strong mobility in terrestrial environments due to their strong water-solubility and are easier to be transported into surface water and groundwater through both leaching and runoff (Picó and Andreu 2007). This can explain why among the three classes of antibiotics, SAs showed the lowest level in soils in the present study, with the total concentrations of SAs (∑SAs) ranging from <LOD to 5.05 μg/kg.

The spatial distribution of antibiotics showed similar spatial variations with the wastewater irrigation type and history of four districts. As shown in Fig. 2, the highest mean concentration (in μg/kg) of ∑FQs and ∑MCs were both found in Dongli (51.79, 2.40), followed by Xiqing (37.94, 1.05) and Jinnan (28.31, 0.34), while the lowest one was found in Beichen (13.56, 0.14). ∑SAs is slightly different with the above two groups of antibiotics. The highest one was found in Xiqing (0.53), followed by Dongli (0.43), Jinnan (0.24), and Beichen (0.13). As mentioned above, the highest antibiotics were found in Dongli or Xiqing, where there is longer-term wastewater irrigation, larger pure wastewater irrigation areas and sewage sludge is used as fertilizer. The antibiotics showed the lowest level in Beichen, where there is only shorter-term intermittent wastewater irrigation. So, wastewater irrigation and sewage sludge used as fertilizer may result in the levels of selected antibiotics increase. That can be a

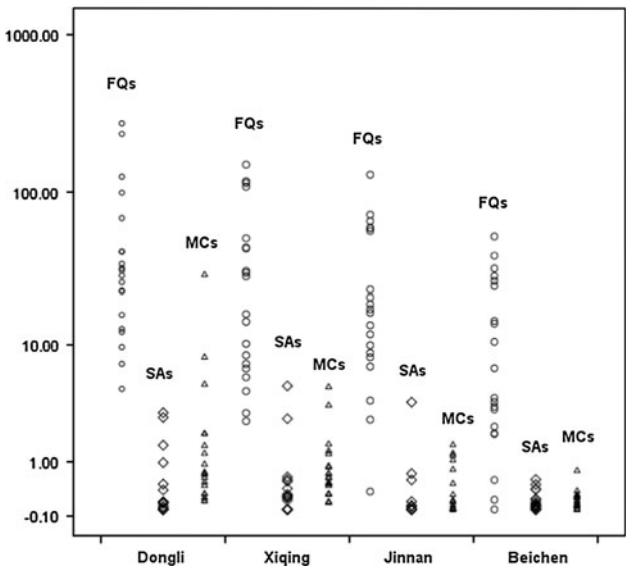

Fig. 2 Concentration (μg/kg) of total FQs, MCs and SAs in soils from four districts of Tianjin. *open circle* concentrations of total FQs in soils from four districts; *open diamond* concentrations of total SAs in soils from four districts; *open triangle* concentrations of total MCs in soils from four districts

potential source of antibiotics in the four districts. In the following, the spatial distribution of individual FQs was discussed and compared with other studies, while individual SAs and MCs were only discussed with the whole data due to their low detection frequency and low concentration.

If all samples were included, among the FQs, NOR was the predominant compound, with the mean concentration (in μg/kg) of 10.22, followed by CIP (6.44), OFL (4.64), FLE (4.34), ENR (4.19), LOM (1.93), SAR (1.11) and DIF (0.69). But there was a slight difference with different districts for the order of FQs. As shown in Fig. 3, the mean concentration of individual FQs were in the order of NOR > CIP > OFL > FLE > ENR > LOM > SAR > DIF for Dongli and Xiqing, NOR > ENR > CIP > FLE > OFL > LOM > DIF > SAR for Jinnan, and NOR > CIP > ENR > OFL > FLE > LOM > SAR > DIF for Beichen. But NOR was the predominant compound in the four districts, ranging from 1.17 to 83.92 for Dongli, from 0.82 to 82.12 for Xiqing, from <LOD to 32.49 for Jinnan, and from <LOD to 12.40 for Beichen. The detection frequencies of NOR were 100 % for Dongli and Xiqing, 86.4 % and 85 % for Jinnan and Beichen, respectively. CIP was the second one in the Dongli, Xiqing, and Beichen, with the mean (range, in μg/kg) concentration of 9.47 (0.57–84.85), 8.32 (0.32–54.45), and 2.36 (<LOD to 7.12), respectively. In Jinnan, the mean CIP level (5.10) is slightly lower than ENR (5.42). As indicated by Tamtam et al. (2011), norfloxacin is a widely used FQ on the market since the late 80 s. And NOR, CIP, OFL and ENR are the main FQ antibiotics in China (Tong et al. 2011). In addition, Picó

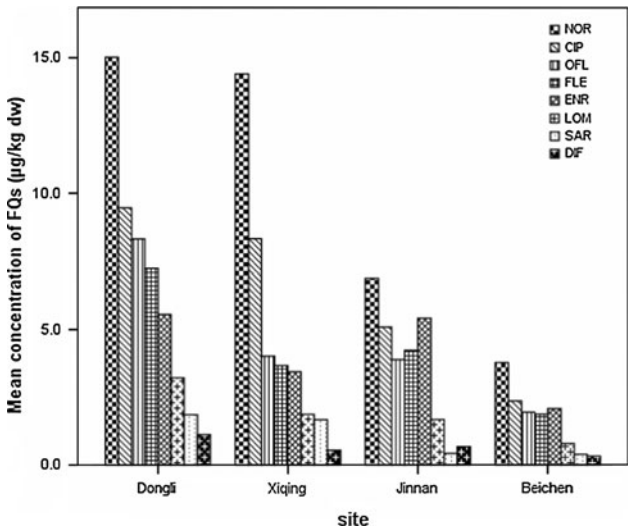

Fig. 3 Concentrations of individual FQs in four districts

and Andreu (2007) reported that CIP, ENR and OFL, which have high sorption coefficient K_d values (61,000, 496–3,037, 44,100 L/kg, respectively), can move rapidly from the water compartment into solids due to their lipophilicity. This may be a potential reason that they showed higher level in soil in the present study. And Golet et al. (2003) also reported that NOR and CIP were found in such a predominant immobilization in soils amended with sludge, with the concentrations of 400 μg/kg.

For the spatial distribution, the highest mean FQs levels were all found in Dongli and the lowest ones were found in Beichen although there were different with different FQ. For NOR, CIP, OFL, LOM, SAR, the highest mean concentrations (in μg/kg) were found in Dongli (1.85–15.02), followed by Xiqing (1.67–14.41), Jinnan (0.43–6.88) and Beichen (0.40–3.78). And for DIF, ENR and FLE, the highest mean concentrations (in μg/kg) were found in Dongli (1.12–7.24), followed by Jinnan (0.68–5.42), Xiqing (0.55–3.44) and Beichen (0.33–2.08). The above difference may result from the different pollution of antibiotics in the irrigation water. So the result showed there may be the most serious antibiotics pollution in Bei Tang Ditch, which was used as irrigation water in Dongli.

Among the sulfonamides, SDMD was mostly detected with the detection frequency of 48 % and the highest level of 3.49 μg/kg. The detection frequencies of other sulfonamides antibiotics were lower than 30 %, with the concentrations of lower than 2.93 μg/kg. Among the macrolides, ROX was mostly detected, with the detection frequency of 72 %. But its levels were lower than 8.16 μg/kg (D15), except one individual sample, whose concentration was 29.20 μg/L at D16 in the Dongli. Maybe there was point source input of macrolide at D16. The detection frequencies of other macrolides were ranging from 16 % to 38 %, with the level lower than 1.55 μg/kg. The antibiotics levels in manure-irrigation soil, reported in some previous studies, usually exhibited higher than ones given in the present study. Li et al. (2011) reported the mean concentration (in μg/kg) of ENR (99.4), NOR (61.9), CIP (26.9) and LOM (7.4) in soil from vegetable farmlands, some of which were fertilized with manure, and the mean concentration of SAs, including SMR, SDZ, SDM, SMX, and SDMD, were reported in the range of 4.9–114.8 μg/kg. In Turkey, ENR was reported at 20–50 μg/kg in the soil seven months after fertilized with manure, indicating the high stability of ENR in soils, and SAs were up to 400 μg/kg due to high concentration in manure (Karci and Balcioğlu 2009). Martínez-Carballo et al. (2007) reported that ENR and CIP were observed in the soil fertilized with manure, with the concentration of up to 370 μg/kg, but SAs were not found in soil sample despite high levels in manure.

As mentioned above, wastewater irrigation may be important potential source of antibiotics. Water in Haihe River, receiving untreated wastewater or effluents from wastewater treatment plants, is mainly used to irrigate in Tianjin. Up to now, there were a few reports on the occurrence of antibiotics in water and sediment of Haihe River. For instance, Zhou et al. (2011) reported FQs levels in sediment from Haihe River were significantly higher than those of SAs and MCs ($p < 0.05$) and the predominant FQ was NOR, with the maximum (median) concentration (in μg/kg) of 5,770 (32.0), followed by CIP (1,290, 16.0), OFL (653, 10.3), LOM (298, 1.67) and ENR (2.34, not detected). However in another report on the occurrence of antibiotics in Haihe River, SAs were the predominant antibiotics in river water and for FQs, only CIP, OFL and ENR included, CIP and OFL were detected with the highest concentrations of 340 and 120 μg/kg for sediment, but ENR was not detected (Luo et al. 2011). The similar concentration pattern is favorable evidence that wastewater irrigation was one of antibiotics sources in soil of Tianjin. Furthermore, Spearman's rank correlation coefficients were calculated to determine the correlation between the levels of fluoroquinolones. The result showed there are significant relations between any two individual FQs ($p < 0.005$), indicating the similar source of FQs.

The Steering Committee of the Veterinary International Committee on Harmonization (VICH) set 100 μg/kg as the trigger value of antibiotics in soil, based on the ecotoxic effects of antibiotic compounds on a range of organisms. The mean concentrations of antibiotics in the present study were all below the trigger value, indicating the low risk for organisms in the agricultural soils of Tianjin. However, it needs to be further studied whether the residues in agricultural soils can risk contamination of the food chain.

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References

Golet EM, Xifra I, Siegrist H, Alder AC, Giger W (2003) Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ Sci Technol 37: 3243–3249

Hu X, Zhou Q, Luo Y (2010) Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut 158:2992–2998

Jimenez B, Chavez A (2008) Water reclamation and reuse around the world. In: Jimenez B, Asano T (eds) Water reuse: an international survey of current practice issues and needs. IWA Publishing, London

Karci A, Balcioğlu IA (2009) Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Sci Total Environ 407:4652–4664

Knapp CW, Dolfing J, Ehlert PAI, Graham DW (2010) Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol 44:580–587

Li YW, Wu XL, Mo CH, Tai YP, Huang XP, Xiang L (2011) Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the Pearl River Delta area, Southern China. J Agric Food Chem 59:7268–7276

Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez PJJ (2011) Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River basin, China. Environ Sci Technol 45:1827–1833

Martínez-Carballo E, González-Barreiro C, Scharf S, Gans O (2007) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ Pollut 148:570–579

McArdell CS, Molnar E, Suter MJF, Giger W (2003) Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. Environ Sci Technol 37:5479–5486

Picó Y, Andreu V (2007) Fluoroquinolones in soil-risks and challenges. Anal Bioanal Chem 387:1287–1299

Shi R, Lv J, Cai Y, Liu Y, Wang Z, Feng J (2010) Levels, spatial distribution and possible sources of heavy metals contamination of suburban soils in Tianjin, China. Bull Environ Contam Toxicol 85:287–290

Tamtam F, Oort F, Bot BL, Dinh T, Mompelat S, Chevreuil M, Lamy I, Thiry M (2011) Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term waste water irrigation. Sci Total Environ 409:540–547

Tong C, Zhou X, Guo Y (2011) Occurrence and risk assessment of four typical fluoroquinolone antibiotics in raw and treated sewage and in receiving waters in Hangzhou, China. J Agric Food Chem 59:7303–7309

Zhou L, Ying G, Zhao J, Yang J, Wang L, Yang B, Liu S (2011) Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River Hai River and Liao River in Northern China. Environ Pollut 159:1877–1885